

(12) UK Patent Application (19) GB (11) 2 402 260 (13) A
(43) Date of A Publication 01.12.2004

(21) Application No: 0312447.6

(22) Date of Filing: 30.05.2003

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(51) INT CL⁷:
H01J 49/42

(52) UK CL (Edition W):
H1D DMD DMG D12A D12C D12E D18L2 D21B D21D
D51

(56) Documents Cited:
GB 2297192 A EP 1298700 A2
WO 2002/078046 A3 US 4978852 A

(58) Field of Search:
UK CL (Edition W) H1D
INT CL⁷ H01J
Other: Online: WPI, EPODOC, JAPIO, INSPEC

(54) Abstract Title: **All-mass tandem mass spectrometry using an electrostatic trap**

(57) A method of obtaining a mass spectrum of elements in a sample is disclosed. Sample precursor ions having a mass-to-charge ratio M/Z are generated, and fragmented at a dissociation site, so as to produce fragment ions of mass to charge ratio m/z . The fragment ions are guided into an ion trap 130 of the electrostatic or 'Orbitrap' type, the fragment ions entering the trap in sub-groups dependent upon the precursor ions' M/Z . The mass-to-charge ratio of each sub-group is determined from the axial movement of ions in the trap. The electric field in the trap is then distorted. Ions of the same m/z , that are derived from different pre-cursor ions, are then separated because the field distortion 202 causes the axial movement to become dependent upon factors other than m/z alone.

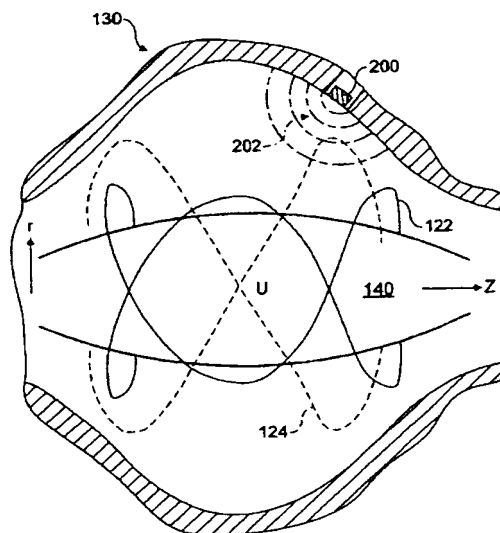


FIG. 3

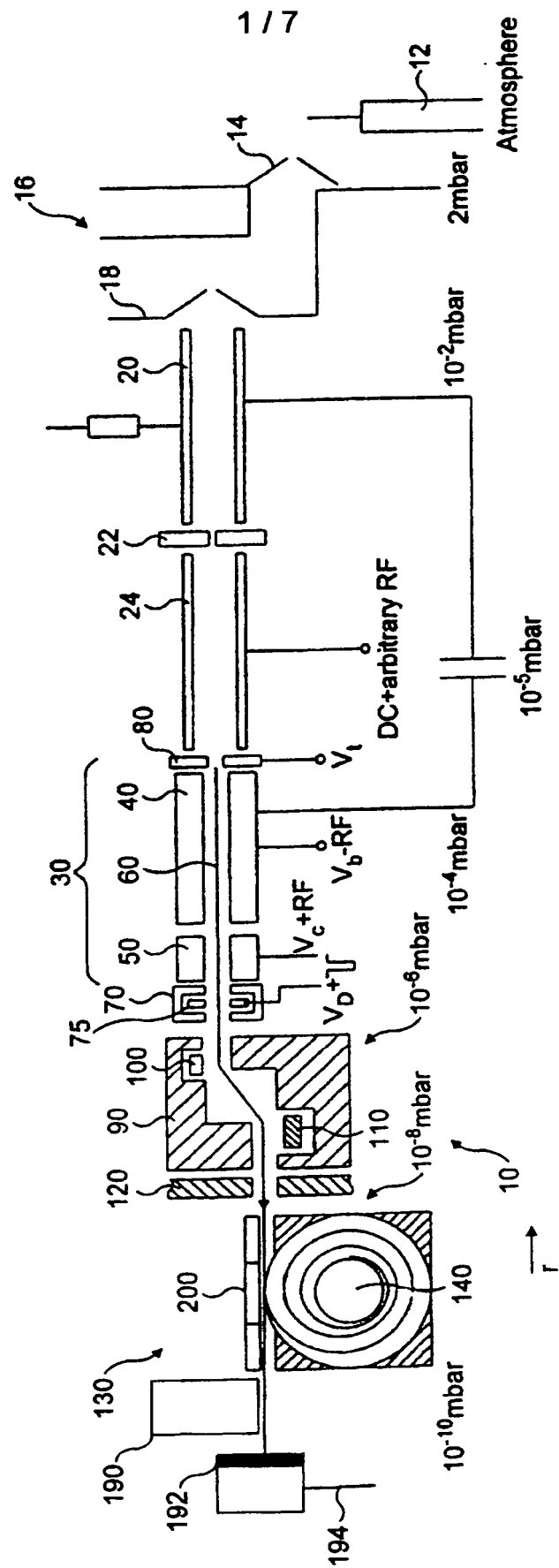


FIG. 1

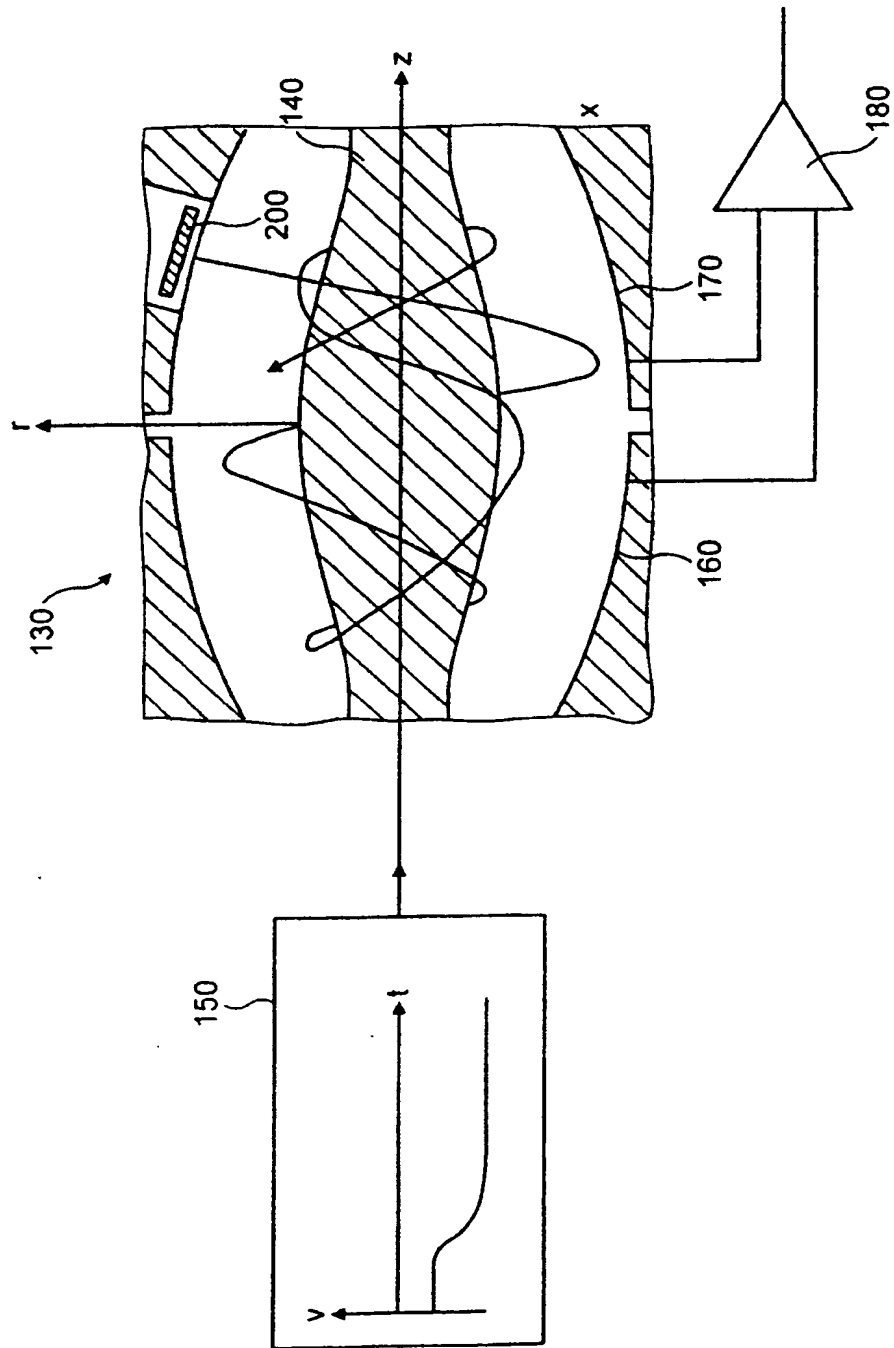


FIG. 2

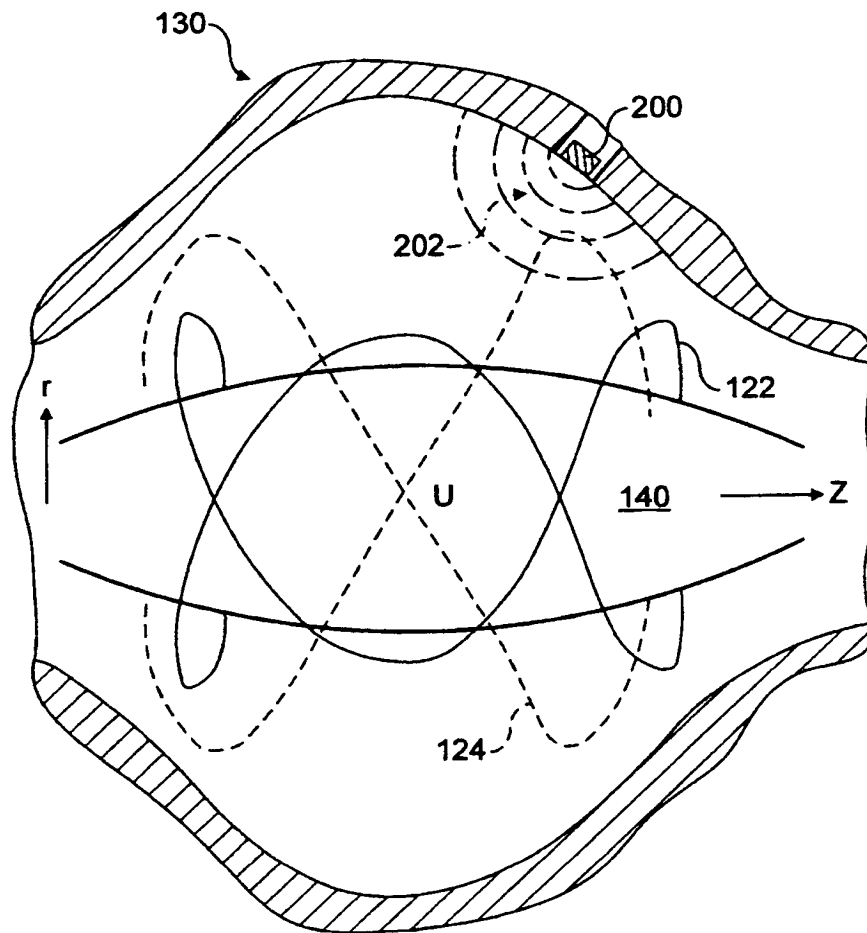


FIG. 3

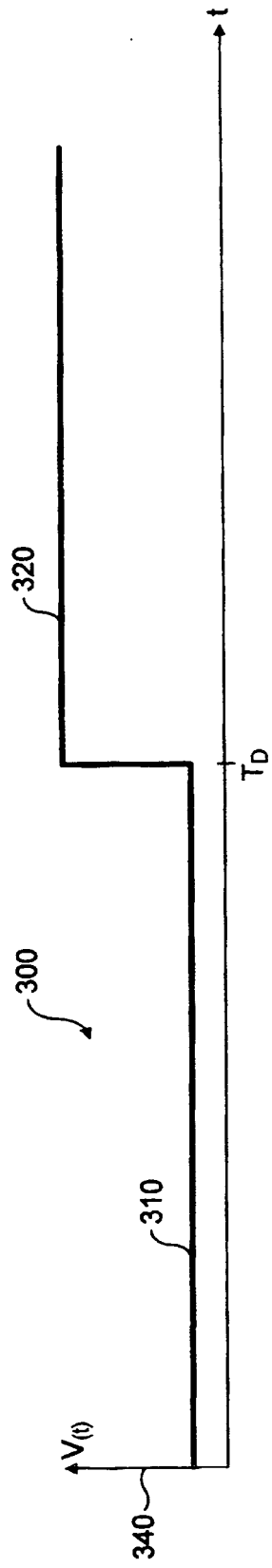


FIG. 4

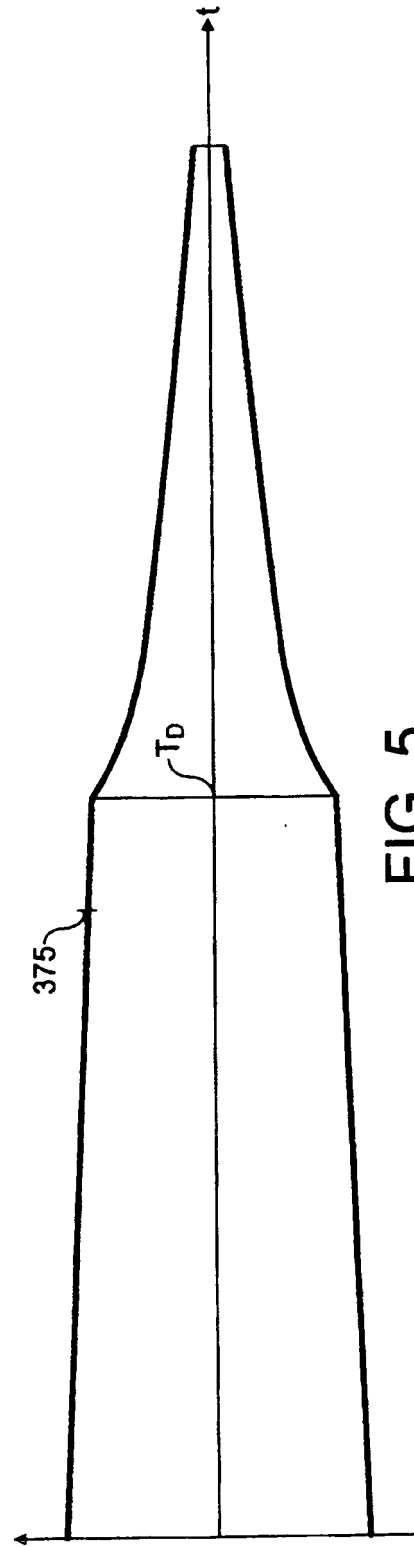


FIG. 5

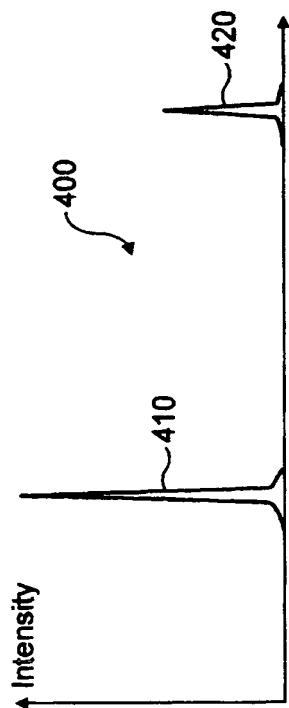


FIG. 6

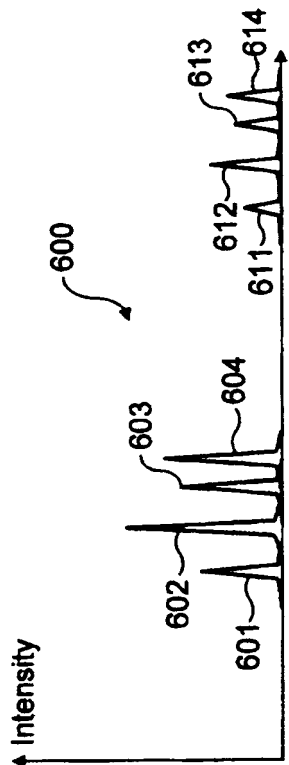


FIG. 8

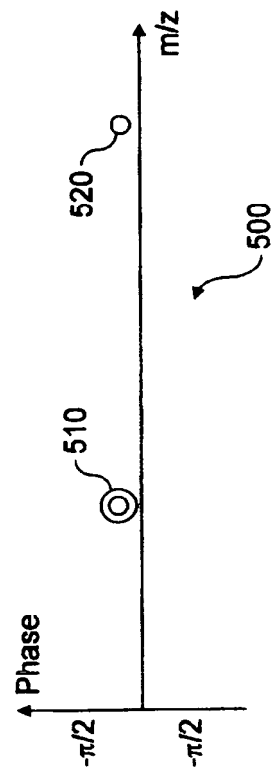


FIG. 7

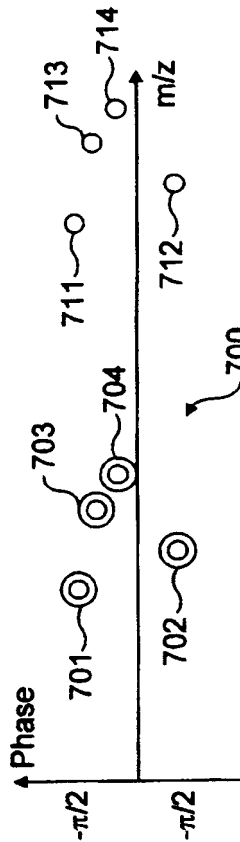


FIG. 9

6 / 7

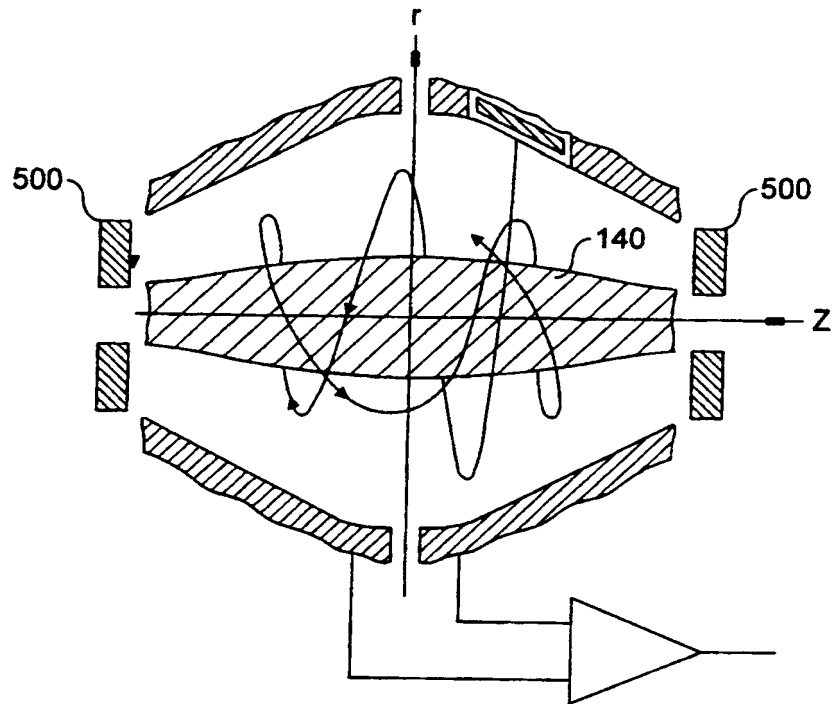


FIG. 10

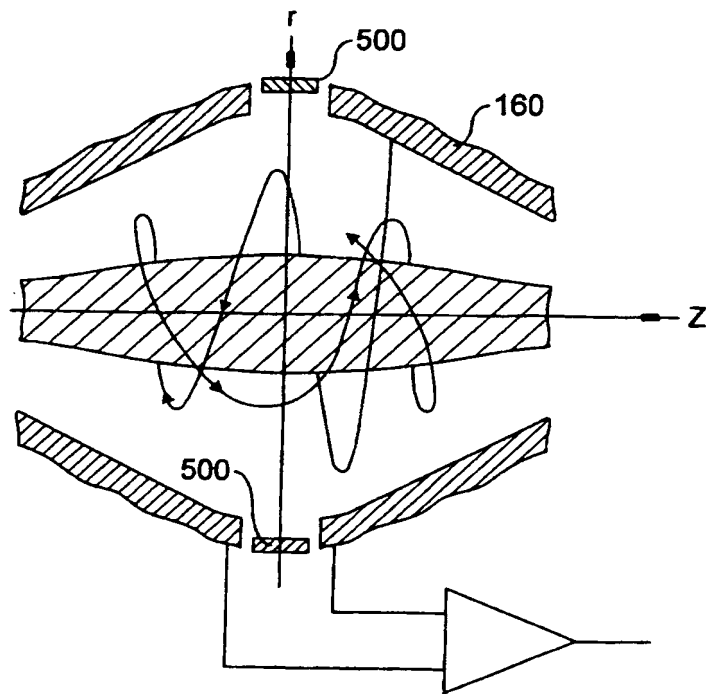


FIG. 11

7/7

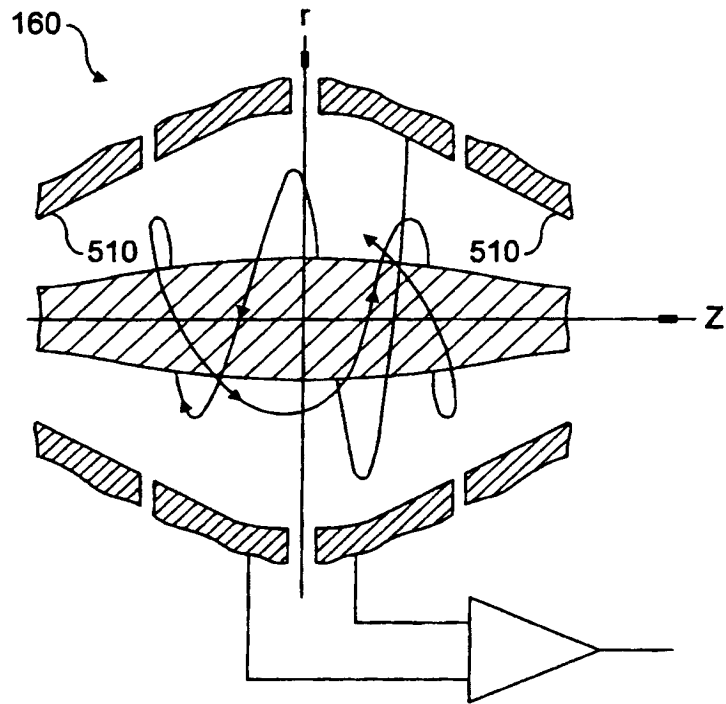


FIG. 12

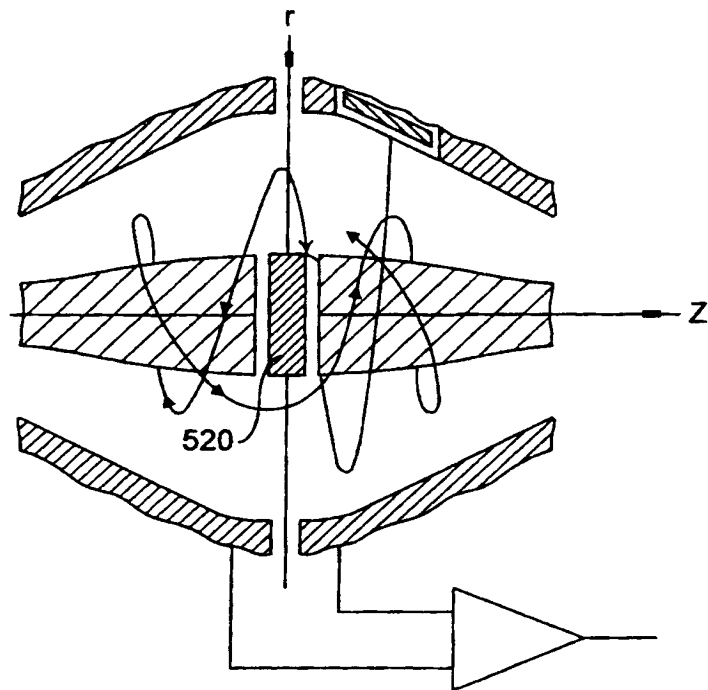


FIG. 13

ALL-MASS MS/MS METHOD AND APPARATUS**FIELD OF THE INVENTION**

5 This invention relates to a method and apparatus of mass spectrometry, and in particular all-mass MS/MS using Fourier Transform electrostatic ion traps.

BACKGROUND OF THE INVENTION

10 Tandem mass spectrometry, or MS/MS, is a well known technique used to improve a spectrometer's signal-to-noise ratio and which can provide the ability to unambiguously identify analyte ions. Whilst
15 the signal intensity may be reduced in MS/MS (when compared with single stage MS techniques), the reduction in noise level is much greater.

 Tandem mass spectrometers have been used to analyse a wide range of materials, including organic
20 substances such as pharmaceutical compounds, environment compounds and biomolecules. They are particularly useful, for example, for DNA and protein sequencing. In such applications there is an ever increasing desire for improving the analysis time. At
25 present, liquid chromatography separation methods can be used to obtain mass spectra of samples. LC techniques often require the use of "peak-parking" to obtain full spectral information and there is a general consensus among persons skilled in the art
30 that the acquisition time needed to obtain complete information about all peaks in a mass spectrum adds a significant time burden to research programs. Thus, there is a desire to move to higher throughput MS/MS.

 Structure elucidation of ionised molecules can be
35 carried out using tandem mass spectrometry, where a precursor ion is selected at a first stage of analysis or in a first mass analyser (MS1). This precursor ion

is subjected to fragmentation, typically in a collision cell, and fragment ions are analysed in a second stage analyser (MS2). This widely used fragmentation method is known as collision induced dissociation (CID). However, other suitable dissociation methods include surface induced dissociation (SID), photo-induced dissociation (PID) or metastable decay.

Presently, there are several types of tandem mass spectrometer geometries known in the art in various geometric arrangements, including sequential in space, sequential in time, and sequential in time and space.

Known sequential in space geometries include magnetic sector hybrids, of which some known systems are disclosed in Tandem Mass Spectrometry edited by W F McLafferty and published by Wiley Inter-Science, New York, 1983; quadrupole time-of-flight (TOF) spectrometer described by Maurice et al in Rapid Communications in Mass Spectrometry, 10 (1996) 889-896; or TOF-TOF described in US 5,464,985. As described in Hoagland-Hyzer's paper, Analytical Chemistry 72 (2000) 2734-2740. First TOF analyser could be replaced by a separation device based on a different principle of ion mobility. The relatively slow time-scale of precursor ion separation in ion mobility spectrometer allows the acquisition of a number of TOF spectra over each scan. If fragmentation means are provided between ion the mobility spectrometer and TOF, then all-mass MS/MS becomes possible, albeit with very low precursor ion resolution.

Sequential in time mass spectrometers include ion traps, such as the Paul trap described by March et al in Quadrupole Storage Mass Spectrometry published by John Wiley, Chichester, 1989; or FTICR spectrometer as described by A G Marshall et al, Optical and Mass Spectrometry, Elsevier, Amsterdam 1990; or LT

Spectrometers such as the one disclosed in US 5,420,425.

Known sequential in time and space spectrometers include 3D trap-TOF (such as the one disclosed in WO 99/39368 where the TOF is used only for high mass accuracy and acquisition of all the fragments at once); FT-ICR such as the spectrometer disclosed by Belov et al in Analytical Chemistry, volume 73, number 2, January 15th 2001, page 253 (which is limited by the slow acquisition time of the MS2); or LT-TOF spectrometers, (for example as disclosed in US 6,011,259, which transmits only one precursor ion but which the inventors claim to have achieved a 100% duty cycle).

All of these existing mass spectrometers are only able to provide sequential analysis of MS/MS spectra, that is, one precursor mass at a time. Put another way, it is not possible to provide an all-mass spectra for all precursor masses in a single analysis using these existing mass spectrometers. Insufficient dynamic range and acquisition speed of MS-2 mass spectrometers are considered to be a limiting factor in the spectrometer's ability.

This dynamic range and acquisition speed problem has been partially addressed for Fourier Transform ion cyclotron resonance (FTICR) mass spectrometers, as described in Analytical Chemistry, 1990, 62, 698-703 (Williams E R et al) and in the Journal of the American Chemical Society, 115 (1993) 7854, Ross C W et al. Two different multiplex approaches have been demonstrated which take advantage of a multi-channel arrangement. These are as follows:

Two Dimensional Hadamard/FTICR mass spectrometry

In this method, a sequence of linearly independent combinations of precursor ions are selected for fragmentation to yield a combination of

fragment mass spectra. Encoding/decoding of the acquired "masked" spectra is provided by Hadamard transform algorithms. Williams E R et al (referred to above) have shown that for N different precursor ions, a given signal to noise ratio could be achieved in experiments having a reduced spectra acquisition time of N/4-fold.

Two Dimensional Fourier/FTICR mass spectrometry

This method uses an excitation waveform to excite all the precursor ions. This provides different excitation states for different masses of precursor ions. Using stored waveform inverse Fourier Transform (SWIFT) methods, the excitation waveform is a sinusoidal function of precursor ion frequency, with the frequency of the sinusoidal function increasing from one acquisition to another. As a result, the intensities of fragment ions for a particular precursor ion are also modulated according to the applied excitation. Inverse 2D Fourier Transform applied to a set of transients results in a 2D map which unequivocally relates fragment ions to their precursors.

According to Marshall A G (referred to above) the first method requires substantially less data storage and the second method requires no prior knowledge of the precursor ion spectrum. However, in practical terms, both methods are not compatible with commonly used separation techniques, for instance HPLC or CE. This is due to the relatively low speed of FTICR acquisition (which is presently no faster than a few spectra per second), and a relatively large number of spectra required. Also, unless the LC separation method is artificially "paused" using relatively cumbersome "peak parking" methods, the analyte can exhibit significant intensity changes within a few seconds (in the most widely used separation methods).

Further, the use of peak parking methods can greatly increase the time to acquire spectra.

GB0126764.0 and WO/02/078046 describes a mass spectrometer method and apparatus using an electrostatic trap. A brief description is provided of some MS/MS modes available for this arrangement. However, it does not address any problems associated with all-mass MS/MS analysis in the trap. The precursor ions are ejected from a storage quadrupole, and focussed into a coherent packet by TOF focussing so that the ions having the same m/z enter the electrostatic trap at substantially the same moment in time.

The trajectories of ions in an electrostatic trap are described by Makarov in "Electrostatic Axially Harmonic Orbital Trapping: A High Performance Technique of Mass Analysis", Journal of Analytical Chemistry, v.72, p1156-1162 (2000). From the equations of motion presented in Makarov's paper, it follows that the axial frequency is independent of the energy and the position of ions in the trap (or phase of ions as they enter to trap). Thus, the axial frequency of ion motion is used for mass analysis.

SUMMARY OF THE INVENTION

The present invention provides a method of obtaining a mass spectrum of elements in a sample, the method comprising: a) generating a plurality of precursor ions from the sample, each ion having a mass to charge ratio of M/Z within a range of mass to charge ratios; b) causing the plurality of precursor ions to move from a location to a dissociation site; c) generating a plurality of fragment ions from the precursor ions at the dissociation site such that each of the fragment ions has a mass to charge ratio of m/z within a range of mass to change ratios; d) causing

the plurality of fragment ions to enter an ion trap, such that the ions enter the trap in sub-groups depending on the precursor ion's M/Z ; e) determining M/Z or m/z of each sub-group from the axial movement of ions in the trap; and f) distorting the electric field in the trap so as to separate ions of the same m/z but derived from different pre-cursor ions within the electrostatic trap.

Preferably, the trap is an electrostatic trap. Advantageously, the method can distinguish two or more fragmented ion sub-groups having the same m/z , each being derived from different precursor ion sub-groups with different M/Z , from one another when the electric field is distorted. The distortion cause the frequency of (axial) oscillation of one ion sub-group to change relative to the other ion sub-group. Thus, where the two ion sub-groups were previous undistinguishable from one another, their change of axial frequency relative to each other now renders them distinguishable. The location might be either the location of ion formation (for instance, if MALDI ion sources are used), or the location from ions are released from intermediate storage in an RF trapping device, for example.

It is possible to "label" each ion sub-group derived from different precursor ions because any one of the parameters (e.g. amplitude of movement of each sub-group in the electrostatic trap, or ion's energy in each sub-group, or the initial phase of oscillation of each sub-group in the electrostatic trap) are dependent on T , in the electrostatic trap* (where T is the TOF of an ion from its place of release to the electrostatic trap entrance), and T is in turn dependent on M/Z or m/z .

The method has further advantages of being able to acquire a full spectrum for each of the many precursor ions in one individual spectrum, if for

example, detection is performed in the electrostatic field using image current detection methods.

5 Determination of the differences of movement amplitude and energies for each of the fragmented ion sub-groups can be achieved by distorting the field in the electrostatic trap. In this way, the axial frequency of trajectories for each of the fragment ions (having the same m/z) in the trap is no longer independent of ion parameters.

10 Preferably the field is distorted locally by applying a voltage to an electrode. The field distortion can be arranged such that the axial oscillation frequency of a fragmented ion relatively close to the distortion is different to the axial
15 oscillation frequency of the other fragmented ion, relatively distant from the distortion. Thus, fragment ions with the same m/z , but being derived from precursor ions with different mass to charge ratios can be distinguished from one another. A
20 method for all-mass MS/MS is therefore achieved.

Embodiments of the present invention are capable of improving the speed of analysis by five to ten times, at least, compared to LC peak parking techniques.

25 The present invention also provides a mass spectrometer comprising an ion source arranged to supply a plurality of sample ions to be analysed, each of which has a mass-to-charge ratio M/Z , an electrostatic trap having an electrostatic trap
30 entrance arranged to receive ions released from the ion source as a convolution of bunched time of flight distributions for each m/z , the electrostatic trap further comprising a plurality of trapping electrodes arranged to trap ions received through the
35 electrostatic trap entrance there between so that the said trapped ions describe movement having periodic oscillations in at least one direction, and at least

one field distorting electrode arranged to provide a localised distortion of the field between the trapping electrodes, so that ions of the same m/z , but which entered the trap at different moments in time, are distinguishable from one another.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is now described by way of example, and with reference to the following drawings, in which;

Figure 1 is a schematic diagram of an apparatus used by the present invention;

Figure 2 is a schematic diagram showing details of the electrostatic trap shown in Figure 1;

Figure 3 is a schematic diagram showing the orbital paths of two ions having the same m/z , but different energy;

Figure 4 is a schematic diagram showing the variation of voltage applied to an electrode over time;

Figure 5 is a schematic diagram showing the envelope of a detected transient ion in the orbitrap;

Figure 6 is a schematic diagram of a mass spectrum acquired before T_0 using embodiments of the present invention;

Figure 7 is a schematic diagram showing a mass spectrum relating to the spectrum of figure 6, except that the phase of each peak detected is shown;

Figure 8 is a mass spectrum acquired after T_0 using an embodiment of the present invention;

Figure 9 is a schematic diagram showing the mass spectrum of figure 8, except that the phase of each peak detected is shown; and

Figure 10 to 13 each show various alternative arrangements of an electrostatic trap embodying the present invention.

We have realised that Fourier Transform mass spectrometers have the potential for acquiring an MS/MS spectrum from multiple precursor ions in a single scan, which can greatly reduce the time burden
5 on acquiring a spectrum to a level at least comparable with, or better than LC.

The present invention is described with reference to an electrostatic trap according to the trap disclosed in GB 0126764.0, WO96/30930 and Makarov's
10 paper (referred to previously) and these documents are hereby incorporated by reference. Reference is made to this trap throughout the description as an "orbitrap". Of course, other arrangements of electrostatic traps can be used and this invention is
15 not limited to use with the specific embodiment disclosed herein and in these reference. The other electro-static traps might include arrangements of multi-reflecting mirrors of planar, circular, elliptical, or other cross-section. In other words,
20 the present invention could be applied to any electrode structure sustained at high vacuum which provides multiple reflections and isochronous ion motion in at least one direction. It is not necessary to describe the orbitrap in great detail in this
25 document and reference is made to the documents cited above in this paragraph. The present invention may also, in principle, be applied to a traditional FTICR, although this would require development of sophisticated ion injection and excitation techniques.
30 For example, some electrodes of the FTICR cell, particularly the detection electrodes, could be energised to provide controlled field perturbation.

Preferably, for accurate detection to take place, the orbitrap requires ions to be injected into the
35 trap with sufficient coherence to prevent smearing of the ion signal. Thus, it is necessary to ensure that groups of ions of a given mass to charge ratio m/z

arrive as a tightly focussed bunch at, or adjacent to, the electrostatic trap entrance. Such bunches or packets are ideally suited for electrostatic traps because the full width half maximum (FWHM) of each of the ion packet's TOF distribution (for a given m/z) is less than the period of oscillation of sample ions having m/z when in the electrostatic trap. Reference is made to US 5,886,346 and GB 0126764.0 which describes particular restrictions on the release potential and these two documents are hereby incorporated by reference. Alternatively, a pulsed ion source (for example using short laser pulses) can be employed with similar effect.

Referring to Figure 1, a mass spectrometer 10 is shown. The mass spectrometer comprises a continuous or pulsed ion source 12, such as an electron impact source, an electrospray source (with or without a Collision RF multiple), a matrix assisted laser desorption and ionization (MALDI) source, again with or without a Collision RF multiple, and so forth. In Figure 1 an electrospray ion source 12 is shown.

Nebulised ions from the ion source 12 enter an ion source block 16 having an entrance cone 14 and an exit cone 18. As is described in WO 98/49710, the exit cone 18 has an entrance at 90° to the ion flow in the block 16 so that it acts as a skimmer to prevent streaming of ions into the subsequent mass analysis components.

A first component downstream of the exit cone 18 is a collisional multipole (or ion cooler) 20 which reduces the energy of the sample ions from the ion source 12. Cooled ions exit the collisional multipole 20 through an aperture 22 and arrive at a quadrupole mass filter 24 which is supplied with a DC voltage upon which is superimposed an arbitrary RF signal. This mass filter extracts only those ions within a window of M/Z interest and the chosen ions are then

released into linear trap 30. The ion trap 30 is segmented, in the embodiment shown in Figure 1, into an entrance segment 40 and an exit segment 50. Though only two segments are shown in Figure 1 it is understood that three or more segments could be employed.

As is familiar to those skilled in the art, the linear trap 30 may also contain facilities for resonance or mass selective instability scans, to provide data dependant excitation, fragmentation or elimination of selected mass to charge ratios.

Downstream of the exit electrode is a deflection lens arrangement 90 including deflectors 100, 110. The deflection lens arrangement is arranged to deflect the ions exiting trap 30 in such a way that there is no direct line of sight connecting the interior of the linear trap 30 with the interior of an electrostatic orbitrap 130, downstream of the deflection lens arrangement 90. Thus, streaming of gas molecules from the relatively high pressure linear trap into the relatively low pressure orbitrap 130 is prevented. The deflection lens arrangement 90 also acts as a differential pumping aperture. Downstream of the deflection lens arrangement is a conductivity restrictor 120. This sustains pressure differential between the orbitrap 130 and the lens arrangement 90.

Ions exiting the deflection lens through the conductivity restrictor arrive at an SID surface 192, on the optical axis of the ion beam from the transfer lens arrangement 90. Here, the ions collide with the surface 192 and dissociate into fragment ions having a mass charge ratio of m/z . The fragment ions, and any remaining precursor ions are reflected from the surface and arrive at the orbitrap entrance. The orbitrap 130 has a central electrode 140 (as may be better seen with reference now to Figure 2). The central electrode is connected to a high voltage

amplifier 150.

The orbitrap also preferably contains an outer electrode split into two outer electrode parts 160, 170. Each of the two outer electrode parts is
5 connected to a differential amplifier 180. Preferably this differential amplifier is maintained at virtual ground.

Referring once more to Figure 1, downstream of the orbitrap is a secondary electron multiplier 190
10 located to the side of the orbitrap 130. Also shown in Figure 1 is an SID surface voltage supply 194. In an alternative embodiment, a deceleration gap can be provided between a grid (placed in front of the CID surface) and the surface. Ions pass through the grid
15 into the gap, where they experience a deceleration force caused by an offset voltage applied to the grid. In this way, the collision energy between the ions and the surface can be reduced in a controlled mannner.

The system, and in particular the voltages
20 supplied to the various parts of the system, is controlled by a data acquisition system which does not form part of the present invention. Likewise, a vacuum envelope is also provided to allow differential pumping of the system. Again this is not shown in the
25 figures although the typical pressures are indicated in Figure 1.

The operation of the system, from ions leaving the ion source 12, entering the segmented linear trap 30, being released from the trap and deflected by the
30 lens arrangement 90 are described in GB 0126764.0. The operation of the system up to release of the ions from a linear trap does not form part of the present invention. Accordingly no further detailed discussion of this aspect of the apparatus is necessary in this
35 document. The operation of the system is hereby incorporated with reference to GB 0126764.0.

The embodiment shown in Figure 1 has the SID

surface placed behind the trap, in a reflective geometry, so that ions pass through the orbitrap without being deflected into the trap entrance (there being no voltage applied to the deflection electrode 200 or electrode 140 at this stage). The ions
5 interact with the collision surface 192, dissociating into fragment ions and are reflected back from the surface into the orbitrap. At this stage, a voltage is applied to the electrode 200 and the ions are
10 deflected into the orbitrap.

The energy of the collisions with the surface (and also the energy spread on the resulting fragments) can be regulated by a retarding voltage 194 applied to the SID surface. The distance between the
15 SID surface and the trap 130 is chosen with ion optical considerations in mind, as well as the required mass range. In the preferred embodiment the ions leave the ion trap 30 and are focused onto the SID surface. As a result, the ions arrive at the SID
20 surface in discrete bunches according to their M/Z. There is no TOF focussing of the precursor or fragment ions from the SID surface into the orbitrap's entrance. The distance L between the SID site and the entrance is preferably between 50-100mm. As a result,
25 the additional broadening of ion packet dL from the SID surface to the orbitrap's entrance is negligible, and typically less than 0.5 to 1mm (as the energy distribution of fragment ions leaving the SID is 10-20 eV and the acceleration voltage is of the order of
30 1keV). The SID is located as close to the orbitrap's entrance as is practical so that any spreading or smearing of ions is minimised. This arrangement is by no means limiting to the invention and other forms of dissociation known in the art may also be used. The
35 principles of reducing smearing by maintaining a short distance between the dissociation site and the orbitrap's entrance remain the same, whatever the form

of dissociation.

The skilled artisan can appreciate that photo-induced dissociation (PID) could be used using an impulse laser. PID utilises relatively high peak power of a pulsed laser to dissociate the precursor ions. The dissociation is preferably made in a region where the precursor ions have lower kinetic energy so that the fragment ions have energies within the energy acceptance of the trap. Furthermore, CID can be carried out in a region of lower kinetic energy of precursor ions, preferably in a relatively short, high pressure collision cell. The cell should be arranged to avoid significant broadening of all the time-of-flight distributions from the linear trap 30. Thus, the time-of-flight of ions inside the CID cell less than, or more preferably, very much less than both the TOF of ions from the linear trap to the cell and from the cell to the orbitraps entrance. At present, we believe that fragmentation by CID is the least preferable because of inherently strict high vacuum limitations of electrostatic traps.

In the operation of the preferred embodiment, a pulse of precursor (or "parent") ions is released from the linear ion trap 30. The ions separate into discrete sub-groups according to their time-of-flights during their transition from the storage quadrupole or sample plate to the dissociation site.

Each sub-group, or packets of ions (which now comprises ions of substantially the same mass to charge ratio) collides with the dissociation site. Here, some precursor ions are fragmented into fragment ions with lower energy (in the order of several eV) than the precursor ion's energy. Fragmentation using SID is essentially an instantaneous process. Thus, the fragment ions are ejected from the dissociation site in sub-groups or packets. These fragmented ion sub-groups have differing TOFs from the dissociation

site to the orbitrap entrance, according to their mass-to-charge ratios. Hence, fragment ions and any remaining precursor ions are injected off axis into the increasing electric field of the orbitrap as
5 coherent sub-groups, depending on their mass-to-charge. Thus, coherent packs for each mass-to-charge ratio of the precursor and fragment ions are formed in the orbitrap.

During ion injection a voltage 150 applied to the
10 central electrode 140 of the orbitrap is ramped. As explained in Makarov's paper (referenced above), this ramping voltage is utilised to "squeeze" ions closer to the central electrode and can increase the mass range of trapped ions. The time constant of this
15 electric field increase is typically 20 to 100 microseconds, but depends on the mass range of the ions to be trapped.

During normal operation, the (ideal) electric field in the orbitrap is hyper-logarithmic, due to the
20 shape of the central and outer electrodes. Such a field creates a potential well along the longitudinal axis direction which causes ion trapping in that potential well provided that the ion incident energy is not too great for the ion to escape. As the voltage
25 applied to the centre of electrode 140 increases, the field intensity increases and therefore the force acting on the ions towards the longitudinal axis increases, thus decreasing the radius of spiral of the ions. As a result, the ions are forced to rotate in
30 spirals of smaller radius as the sides of the potential well increase in gradient.

As discussed in the prior art, there are three characteristic frequencies of oscillation within the hyper-logarithmic field. The first is the harmonic
35 motion of the ions in the axial direction where the ions oscillate in the potential well with a frequency independent of ion energy. The second characteristic

frequency is oscillation in the radial direction since not all of the trajectories are circular. The third frequency characteristic of the trapped ions is the frequency of angular rotation. The moment T of an ion pack entering the orbitrap electric field is a function of m/z or M/Z and is defined in equation 1 provided below:

$$T(m/z, M/Z) \approx t_0 + TOF_1(M/Z) + TOF_2(M/Z) \cdot \sqrt{\frac{m/z}{M/Z}} \quad (1)$$

where t_0 is the moment of ion formation or release from the trap, $TOF_1(M/Z)$ is the time-of-flight of precursor ions from the place of ion release or ion formation to the collision surface, and $TOF_2(M/Z)$ is the time-of-flight of precursor ions from the collision surface to the entrance to the orbitrap, and M/Z and m/z are mass charge ratios of precursor and fragment ions respectively. Ions could also be generated from a solid or liquid surface using MALDI, fast atom bombardment (FAB). Or secondary ion bombardment (SIMS) or any other pulsed ionization method. In these cases, t_0 is the moment of ion formation. The effects of energy release, energy spread and other constants or variables are not included in equation 1 for clarity reasons.

There are parameters which are dependent on ion mass-to-charge ratio due to the separation of the ions into sub-groups according to their TOF from the quadrupole. These parameters include the amplitude of movement during detection in the orbitrap (for example, radial or axial amplitudes), the ion energy during detection, and the initial phase of ion oscillations (which is dependent on T). Any of these parameters can be used to "label" the precursor or

fragment ions.

It is preferable that the fragment ions are formed on a timescale such that TOF effects do not disrupt the fragmented ion package coherence to an extent which might effect detection (eg. because of smearing caused by energy spread). The parameters of the fragment ions may differ from those of the precursor ions. However, the fragment ions can be unequivocally related to their precursor ion's parameters. This is achieved in the following manner.

In a preferred embodiment, detection of the ion's axial oscillation frequencies in the trap starts at a predetermined detection time T_{det} after t_0 . T_{det} is typically several tens of milliseconds (for instance 60ms or more) after t_0 and the TOF of ions from the storage trap is typically 3 to 20 micro seconds (for instance). The period $T_a(m/z)$ of ion axial oscillations is of the order of a few microseconds, depending on the M/Z or m/z , of course. The phase of oscillations $P(m/z, M/Z)$ can therefore be determined using equation 2 below:

$$P(m/z, M/Z) = 2\pi \cdot \text{fraction} \left\{ \frac{T_{det} - T(m/z, M/Z)}{T_a(m/z)} \right\} + c \quad (2)$$

where P is the phase, c is a constant and $\text{fraction}\{\dots\}$ is a function that returns a fractional part of its argument.

According to the Marshall (referenced above) the detected phase, $P_{det}(\omega)$, can be deduced by detecting the adsorption and dispersion frequency spectra, $A(\omega)$ and $D(\omega)$ respectfully, as set out in equation 3 below:

$$P_{\text{det}}(\omega) = \arctan \left\{ \frac{D(\omega)}{A(\omega)} \right\} \quad (3)$$

and using the relation between the axial frequency of motion of ions ω and m/z for the orbitrap

$$\omega(m/z) = \sqrt{k \cdot (m/z)} \quad (4)$$

where k is a constant derived from the orbitrap's electric field. The period of ion oscillations $T_a(m/z)$ is linked to the axial frequency ω as

10

$$T_a(m/z) = \frac{2\pi}{\omega(m/z)} \quad (5)$$

Thus, for a given m/z , and using constants derived from a preliminary system calibration, it is possible to deduce M/Z from equations 1 to 4. In other words, $P(m/z, M/Z)$ is deduced from the measured phase and m/z (using equations 3 and 4) and from these values it is possible to deduce $T(m/z, M/Z)$ from equation 2. As a result, it is possible to deduce M/Z from equation 1. Thus, the ion from which a fragment ion is derived can be accounted for because the axial oscillation of the fragment ion is linked to the phase of the precursor ion oscillation in the orbitrap (assuming that the fragment ion's mass to charge ratio arises from a single precursor ion).

The initial phase of oscillation of the precursor and fragment ions in the orbitrap is dependant on T which can be deduced from, for example, real and imaginary parts of the Fourier Transform of the fragment ion's axial oscillation frequency. Alternatively, T can be measured directly using TOF

spectra acquired by electron multiplier 190. Mass m could then be deduced using an appropriate calibration curve for the orbitrap. In this manner, all-mass MS/MS spectroscopy is achievable.

5 However, the situation can be more complicated if two (or more) precursor ion sub-groups having different M/Z produce a plurality of fragmented ion sub-groups having the same m/z . In any case, if ions of the same m/z (but derived from different precursor
10 ions with different M/Z) enter the orbitrap at different moments in time, their axial oscillation frequencies are the same and so they are not distinguished from each other. This is so because the ion's frequency of axial oscillations are
15 independent of ion energy and initial phase of ion oscillation (i.e. it is only dependent on mass-to-charge ratio).

 This situation can be exemplified as follows. Taking the situation where two groups of precursor
20 ions with mass-to-charge ratios M_1/Z_1 and M_2/Z_2 respectively are released from the ion storage at substantially the same time and where M_1/Z_1 is lighter than M_2/Z_2 . As normal, the ion with the lower mass-to-charge ratio moves faster than the heavier,
25 following

$$TOF(M/Z) \propto \sqrt{M/Z} \quad . \quad (5)$$

As a result, M_1/Z_1 ion arrives at the SID surface
30 earlier than M_2/Z_2 . Here the M_1/Z_1 ions promptly fragment producing, amongst others, a fragment ion with m/z , which starts moving towards the orbitrap's entrance. If, for example, $m/z < M_1/Z_1$ (which is not always the case, for instance when $m < M_1$, but $z \ll Z_1$),
35 then fragment ion m/z overtakes any M_1/Z_1 precursor ions which did not fragment at the SID. Thus,

according to equation 5 above, ions with mass to charge ratio of m/z arrive at the orbitrap's entrance before the unfragmented precursor ions. The time difference of arrival at the entrance is governed by equation 1. It is possible that, while the $M1/Z1$ group of ions are still in transit between the SID and the orbitrap's entrance, the ion group of $M2/Z2$ arrive at the SID. Here they too fragment, forming (amongst others) a second sub-group of ions with a mass to charge ratio of m/z , which proceed to move towards the orbitrap's entrance. As before, ions in the group m/z are likely to "overtake" ions in the group $M2/Z2$ on their way to the orbitrap. The second group of m/z ions to arrive at the orbitrap's entrance after the $M1/Z1$ group. As a result, the sub-group of fragment ions (with mass to charge m/z) arriving at the orbitrap's entrance first has a different phase to a later sub-group with the same mass to charge ratio m/z . (In extreme, and very unlikely, cases the phases of the two fragment ion sub-groups can cancel one another out, resulting in no signal being detected). If the electric field in the orbitrap is ideal (that is hyperlogarithmic) then both sub-groups give a single spectral reading for the same m/z . This is undesirable since it is difficult to attribute from which pre-cursor ion group sub-group with mass to charge ratio m/z is derived. Thus, this signal needs to be unscrambled.

This unscrambling can be achieved by initiating the ramping of the voltage 150 at a time before ions enter the trap, and to terminate the ramp at a time after all the ions of interest have entered the trap. As a result, a first sub-group ions entering the trap at a earlier time than a second sub-group experience more of the ramped voltage than the second sub-group. Thus, the first sub-group of ions are "squeezed" closer to the central electrode than the second group.

As a result the amplitude of oscillation is therefore greater for the second sub-group than the first sub-group. The first and second sub-groups of ions have distinctly different orbital radii about the central electrode.

5 However, because the axial oscillation frequency is used for mass analysis in the orbitrap, and the axial frequency is not dependent on ion energy or radius (or linear velocity as the ions enter the orbitrap), the first and second fragment ion sub-
10 groups have the same axial frequency. As a result, they are not resolved from one another in conventional mass analysis using the ideal E-field. Thus, using a calibration curve to determine the mass M may produce
15 a wrong assignment of the fragmented ion to a precursor ion.

An aspect of the present invention provides a way to assign the fragment ions to their correct precursor ions. This is achieved by assessing differences in
20 amplitudes of movement and energies of the ions in the orbitrap. This can be done by shifting the frequency of oscillation of one sub-group relative to the other (although the frequency of axial oscillations in the orbitrap is normally independent of these parameters.)
25 The "frequency shift" can be introduced by distorting the ideal electric field in the orbitrap in an appropriate manner. Preferably, the distortion is localised, for example, by applying a voltage to a (normally grounded) electrode disposed between, or
30 near, outer detection electrodes.

It is preferable to charge the electrode to an extent that it distorts the field away from the hyper-logarithmic field so that the ions remain trapped, the ions amplitude of movement decays at a rate which does
35 not prohibit efficient detection and the ideal field is distorted so that ions of different energies and/or a sufficient frequency shift is introduced between the

two (or more) sub-groups with the same m/z .

In a preferred embodiment, for trapped ions having energies of a few kilo-electronvolts, a voltage is applied to the deflection electrode 200 to provide localised distortion 202 to the trap field. The voltage is typically between 20 to 250 volts, but may be higher or lower, depending on the energy of ions in the orbitrap. As a result, the detected axial frequency of ions oscillating relatively close to the distortion (that is, the sub-group which entered the orbitrap last, and having a larger orbit radius), is different from ions with the same m/z oscillating further away from the distortion (that is, the sub-group which entered the orbitrap at an earlier time).

With reference to figure 3, a schematic diagram of two ion's orbital paths 122, 124 in an orbitrap 130 are shown. Both the ions have the same m/z . Their oscillation frequencies along the trap's longitudinal axis z are the same when an ideal hyper-logarithmic field is applied to the ions, as discussed previously. Ions in the orbital path 124 have a higher oscillation amplitude or orbital radius than ions in the path 122. From figure 3 it can be seen that when a voltage is applied to the deflection electrode 200, the E field is distorted 202. Of course, the distortion is most intense close to the electrode and diminishes as the distance from the electrode increases. It can be seen that ions in the higher orbital path 124 experience the distorted field to a greater extent than ions in the lower orbital path. Hence, the axial oscillation frequency (and phase) of ions in the higher oscillation amplitude path is affected (and shifted) to a greater extent than oscillation frequencies of ions in lower oscillation amplitude orbital paths. Thus, the detected mass spectrum peaks for ions of the same m/z , but having different precursor ion M/Z , are split into separated, resolvable peaks. Further, the

initial phase of ions associated with each peak are resolvable.

With reference to Figure 4, a voltage applied to the electrode used for introducing the field distortion in the electrostatic trap, with respect to time, is shown. The voltage has two distinct stages, a low voltage stage 310 and a high voltage stage 320. The step 330 at time T_{step} between stage 1 and 2 is relatively rapid so that the field perturbations are introduced almost instantaneously. The voltage scale 340 in figure 4 only shows arbitrary values. The likely time required for each stage is preferably of the order of a few hundred milliseconds to a couple of thousand milliseconds for stage 1 and of the order of a few tens to a hundred milliseconds for stage 2. The transition between stage 1 and 2 should preferably be in the region of 10 microseconds, or so. The voltage applied to the electrode during stage 1 should be chosen such that the field in the orbitrap is not distorted. Hence, if the electrode to which the distortion voltage is to be applied is disposed close to a normally grounded orbitrap electrode, then the initial voltage in stage 1 should also be ground, assuming the distortion electrode is on the same equipotential as the detection electrode.

With reference to figure 5, the amplitude 375 of a sub-group of ions in an orbit in the orbitrap is shown with respect to time. It can be seen that the amplitude decays relatively slowly when the ions are trapped by an ideal E field. However, the amplitude decays at a very much faster rate when the ideal field is distorted after T_D .

Referring to figure 6, a graph 400 of a mass spectrum resolved during stage 1 (that is no field perturbation in the orbitrap) is shown. Two peaks 410 and 420 are shown, each having different intensities and mass to charge ratios m/z . Figure 7 shows a

representation of the spectrum shown in figure 6 where the phase of the two peaks in figure 6 is shown against mass to charge ratio M/Z . The point 510 corresponds with peak 410 in figure 6 and the point 520 corresponds to peak 420 in figure 6.

Since the spectrum shown in figure 6 and 7 are taken during the first acquisition stage, it is not possible to deduce whether any of the points in the spectrum are associated with a single fragmented ion or more than one fragment ions having the same mass to charge ratio (but being derived from different precursor ions of different mass to charge ratios). Referring to figure 8, the same spectrum shown in figure 6 is shown. However, the spectrum 600 in figure 8 is taken during stage two, that is when a voltage is applied to the electrode to distort the electric field in the electrostatic trap 130. The group of peaks 601 to 604 corresponds with the peak associated with 410 of the spectra taken during stage one. Likewise, the group of peaks made up of peaks 611 to 614 are associated with the peak 420 of the spectra taken during stage one. Thus, it can be seen that each of the peaks of the spectra taken in stage one (when the electric field in the electrostatic trap was homogeneous) have four fragment ions associated with each peak taken during stage one.

Figure 9 corresponds with the spectrum shown in figure 8 but the phase of each of the peaks in figure 8 is shown. Points 710 to 714 and points 711 to 714 correspond to peaks 610 to 614 and 611 to 614 respectively. Thus, figures 8 and 9, when compared with figures 6 and 7 respectively, show how the non-homogeneous electrostatic field in the orbitrap can be used to "split" spectrum lines into their constituent lines.

Faster signal decay and the resulting lower resolving power is expected due to the trap's

inhomogeneous electric field, as shown in figure 5. The present method should allow the separation of fragmented or precursor ions whose mass-to-charge ratio are within a few percent of one another. If individual spectral peaks cannot be resolved then the corresponding fragment or precursor ion associated with the peaks can be flagged as unidentifiable.

It is preferable to acquire the data in two stages, as shown in figure 4. In stage one the electrostatic field is maintained at an ideal state (or as close to this ideal as possible) so that the highest possible resolving power and mass accuracy are obtained from the spectrometer. During stage one the masses are measured to a high accuracy and any possible isobaric interferences are also measured.

The system then switches to the second stage in which the electric field is perturbed by applying a voltage to an electrode close to one of the orbitrap electrodes. This perturbation causes spectral peaks to split and thus facilitates fragment assignment. Preferably, the second stage is much shorter than the first stage. Both stage one and two are preferably performed within a single spectrum acquisition.

The embodiments described above are described with reference to electrostatic trap mass spectroscopy. However, the methods may be applicable to other forms of ion mass spectroscopy.

Also, variations of the apparatus and methods described above may also be envisaged by a person skilled in the art without leaving the scope of the claims. For instance, it may be preferable to provide a dedicate E field distortion electrode. This can be disposed on or off the orbitrap's equatorial axis. The electrode for distorting the E field can be disposed at various locations in the orbitrap, some examples of which are shown in figures 10 to 13.

Referring to figure 10, the distorting electrode

500 is arranged as an annular ring electrode at either end of the central electrode 140. With reference to figure 11, the distortion electrode 500 is disposed as an radial ring about the centre of the outer electrode 160. With reference to figure 12, the outer electrode 160 is split into four parts comprising two inner and two outer electrodes. During stage one of a spectral acquisition, all of the outer electrode components can be arranged to operate at the same voltage to produce the ideal E field. However, during stage two, a different voltage is applied to the two outermost electrodes 510 to distort the ideal field. The field distorting electrode 510 should be arranged so that axial oscillations of ions in the ideal field are generally within the inner edge of the distortion electrode. Of course, the distortion electrode may also be applied to the inner electrodes as well. Referring to figure 13, the distorting electrode 520 is disposed on the central electrode. In this example, the distorting electrode is shown at a central position, but it could also be arranged in any convenient location on the central electrode.

Other methods of distorting the electrostatic field will be apparent to skilled persons, other than the electrostatic distortion described above. For instance, resonant excitation of the ions by applying an RF voltage to the electrode would be used to provide a dependence of frequency on the ion's parameters.

Also, the foregoing description refers to TOF ion separation. However, the present invention is not limited to only this method and other forms of ion separation, such as ejection from a linear trap for instance, may be equally appropriate. For example, another embodiment of the present invention may include sequential ejection of precursor ions (which might have monotonously increasing or decreasing M/Z)

towards the dissociation site. Thus, the TOF_1 term in
equation 1 above is replaced with a scan dependent
function. In practice, such a scan could be provided
in different constructions of analytical linear traps,
5 such as those described in US 5,420,425 or WO00/73750.

CLAIMS:

1. A method of mass spectrometry using an ion trap,
the method comprising:
 - 5 a) generating a plurality of precursor ions from the sample, each ion having a mass to charge ratio of M/Z within a range of mass to charge ratios;
 - b) causing the plurality of precursor ions to move from a location to a dissociation site;
 - 10 c) generating a plurality of fragment ions from the precursor ions at the dissociation site such that each of the fragment ions has a mass to charge ratio of m/z within a range of mass to charge ratios;
 - d) causing the plurality of fragment ions to enter an ion trap, such that the ions enter the trap in sub-groups depending on the precursor ion's M/Z ;
 - 15 e) determining at least one of M/Z and/or m/z of each sub-group from the frequency and phase of movement of ions in the trap; and
 - 20 f) distorting the electro-magnetic field in the trap so as to separate ions of the same m/z but which are derived from different pre-cursor ions within the trap.
- 25 2. A method according to claim 1, wherein the ion trap is an electrostatic trap.
3. A method according to claim 1, wherein the ion trap is an FT ICR cell.
- 30 4. A method according to claim 2, wherein the two or more fragmented ion sub-groups are each being derived from different precursor ion sub-groups with different M/Z .
- 35 5. A method according to claim 2 or 4, wherein the electric field in the trap is substantially hyper-

logarithmic prior to step f).

5 6. A method according to claim 5, wherein the two or more fragmented ion sub-groups have a substantially similar initial frequency of axial movement in the trap before the electric field is distorted.

10 7. A method according to claim 2 or 6, wherein the two or more fragmented ion sub-groups have a different phase of axial movement in the trap when the electric field is distorted.

15 8. A method according to claim 1 or 2, wherein the two or more fragmented ion sub-groups are distinguished from one another by determining differences of movement amplitudes and/or frequencies and/or phases and/or energies of each sub-group in the trap.

20 9. A method according to claim 2, wherein the electric field distortion is substantially localised.

25 10. A method according to claim 2 or 9, wherein the field is distorted by applying a voltage to an electrode.

30 11. A method according to any one of claims 2 to 10, wherein the voltage is applied after a predetermined time from the ions being injected into the trap.

35 12. A method according to claim 4, wherein the field distortion is arranged such that the oscillation frequency of the at least two fragmented ion sub-groups are different.

13. A method according to any one of claims 1 to 12, wherein the mass spectrum is obtained in two stages,

in a first stage the trap electro-magnetic field is undistorted, and in a second stage the electro-magnetic field is distorted so that any fragment ions having the same m/z and which are derived from precursor ions with different M/Z can be distinguished from one another.

14. A method according to claim 13, wherein the second phase commences after a predetermined period.

15. A method according to claim 1, wherein the fragment ions are generated by any one of surface induced dissociation (SID), collision induced dissociation (CID), or photon induced dissociation (PID).

16. A method according to claim 15, wherein, when the fragment ions are generated by SID, a retarding voltage is supplied to a collision surface.

17. A method according to claim 16, wherein the collision surface is arranged in a reflection geometry.

18. A method according to claim 15, wherein when the fragment ions are generated the fragmented ions have less kinetic energy with respect to their precursor ions.

19. A method according to claim 15, wherein CID generated fragment ions are generated in a collision cell so arranged that the time-of-flight (TOF) of ions in the cell is less than the TOF of the ions from the location to the cell and from the cell to the trap.

20. A method according to claim 2, wherein the distortion is electrostatic or has an RF component.

21. A mass spectrometer comprising:

an ion source arranged to supply a plurality of sample ions to be analysed, each of which has a mass-to-charge ratio m/z ,

5 an electrostatic trap having an electrostatic trap entrance arranged to receive ions released from the ion source as a convolution of bunched time of flight distributions for each m/z , the electrostatic trap further comprising a plurality of trapping
10 electrodes arranged to trap ions received through the electrostatic trap entrance there between so that the said trapped ions describe movement having periodic oscillations in at least one direction, and

15 at least one field distorting electrode arranged to provide a localised distortion of the field between the trapping electrodes, so that ions of the same m/z , but which entered the trap at different moments in time, are distinguishable from one another.

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:: 170690: CRC: CRC: READDOCS



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Application No: GB 0312447.6
Claims searched: All

Examiner: Geoff Holmes
Date of search: 21 January 2004

Patents Act 1977 : Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance	
A	-	WO 02/078046 A3	[THERMO FINNIGAN] see figures 1 & 3
A	-	EP 1298700 A2	[HD TECHNOLOGIES] see figures 2 & 3
A	-	US 4978852 A	[WILLIAMS et al.] see abstract
A	-	GB 2297192 A	[BRUKER FRANZEN]

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X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
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Field of Search:

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The following online and other databases have been used in the preparation of this search report:

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